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Tomentellopsis pulchella sp. nov. from St. Vitale Pine Forest (Ravenna, Italy)

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Abstract—A new thelephoroid species, *Tomentellopsis pulchella,* is described from Italy. It is a distinct taxon with yellow to orange hymenium and with small globose spores. According to the rDNA ITS sequences the closest species are *T. bresadolana* and an undescribed taxon found in Finland. So far *T. pulchella* is found only in St. Vitale (Italy) pine-forest.

Key words—taxonomy, thelephoroid fungi, *Thelephorales*, ectomycorrhiza

Introduction

The genus *Tomentellopsis* belongs to the fungal order *Thelephorales* (*Homobasidiomycetes*) and all known species of the genus form resupinate basidiomata. Kõljalg (1996) recognized in this genus only three species, viz. *Tomentellopsis echinospora*, *T. pusilla,* and *T. zygodesmoides*. Based on morphology and molecular features we accept here also *T. bresadolana* and *T. submollis,* following in this way the concept of the genus proposed by Hjortstam (1974). The basidiomata of *Tomentellopsis* are mostly found in the upper layer of the soil attached to the underside of dead wood, leaves, and other debris. It is likely that all species are ectomycorrhiza (EcM) formers with worldwide distribution. The well-known pink-type EcM was lately linked via rDNA ITS sequences to *T. submollis* (Kõljalg et al 2002). Soon other studies followed, which showed that *T. submollis*, *T. zygodesmoides* and *T. echinospora* are forming EcM on different host trees (Burke et al. 2005, Rosling et al. 2003, Saari et al. 2005, Tedersoo et al. 2006, Walker et al. 2005). We have no proof that the new species described in this paper forms EcM, but based on previous studies it is logical to deduce this.

Mycobiota of the St. Vitale pine-forest (Ravenna, Italy) is comparatively well studied with many rare species, including some new to science (e.g. Bernicchia 2005, Hausknecht & Zuccherelli 1999, Noordeloos & Hausknecht 2002). In 2000 and 2001 several yellow to orange basidiomata of an unknown *Tomentellopsis* were collected on small branches, twigs and litter, particularly of *Crataegus* and *Quercus*. Based on morphology and molecular analyses we describe these specimens as a new species, *T. pulchella*.

Material and methods

Light microscopy

For light microscopic studies, samples were mounted in 3% potassium hydroxide (KOH) and Melzer's solution. Microphotos were made using an AxioCam MRc digital camera attached to a Carl Zeiss Axioskop 2 microscope and grabbed into computer with AxioVision 3.0 software.

Molecular and phylogenetic analyses

Pieces of hymenium from sporocarps were used for the DNA extraction. The primer pairs ITS1-ITS4 or ITS1F-ITS4B (White et al. 1990, Gardes & Bruns 1993) were used for the amplification and sequencing of ITS regions. For comments on these two primer pairs and for full descriptions of molecular methods see Kõljalg et al. (2002) and Tedersoo et al. (2003). For this study four new sequences were obtained and they were deposited in EMBL as well as in UNITE (Kõljalg et al. 2005). The UNITE accession is linked to a colour picture of the basidioma. The additional 38 ITS sequences used in this study were received from EMBL and GenBank databases.

The ITS sequences were assembled and aligned using Sequencher 4.6 (GeneCodes Corp.). Alignment was checked and improved manually in PAUP* 4.0b10 (Swofford 2003). The final dataset included 44 ITS sequences – 22 sequences originating from ectomycorrhizas and 22 sequences from sporocarps. After alignment the database included 636 characters but forty extreme 3' positions were excluded from all analyses because they were incomplete for many taxa.

Phylogenetic analyses were performed using programs PAUP* 4.0b10 and MrBayes 3.1.2 (Huelsenbeck & Ronquist 2001), all characters were treated as unordered, equally weighted and gaps were treated as missing data. Maximum parsimony (MP) analyses were conducted using 1000 heuristic searches with random taxon addition sequences, TBR branch swapping, maxtrees set to 10000, and restrictions to save 100 trees per replicate. Followed by additional swapping of the resulting trees. A bootstrap analysis was performed with 1000 replicates, each with 10 random taxon addition sequences.

A distance neighbour-joining analysis was conducted with substitution model TrNef+I+G selected by Modeltest 3.7 (Posada & Crandall 1998) under Akaike (AIK) information criterion.

Bayesian inference of phylogeny was performed with MrBayes 3.1.2 with the SYM+I+G substitution model chosen through AIK information criterion in MrModeltest 2.2 (Nylander 2004). The Markov chain Monte Carlo (MCMC) was run with 2000000 generations, and with default values of other prior settings. According to the specified generations the first 200000 generations without reaching a stable likelihood score were discarded, leaving 36002 trees for computing the consensus trees and posterior probability (PP) values.

The difference between samples was determined using the PAUP* command Pairwise Base Difference and are shown as percentages.

Species description

Tomentellopsis pulchella Kõljalg & Bernicchia, **sp. nov.** FIGS. 1–3

MYCOBANK MB 511675

Carposomata effusa, cohaesa deinde pellicularis, subtilia, separabilia. Hymenophorum leve, arachnoideum, gilvum, flavente in maturitate, ochraceum cum exsiccatione; margine leviter fimbriato, rhizomorphae adsunt, filii mycelii aliquando marginibus adsunt. Hyphae subiculi septatae, fibulis vacuae, tenuiter tunicateae vel leviter crassitunicatae, hyalinae aut flavo-brunneae, 4.2–5.5 µm latae. Hyphae subhymeniales hyalinae, leves, 2.8–4.2 µm latae, septatae, ramosae, tenuiter tunicatae. Cystidia desunt. Basidia hyalina, sinuosa, flexuosa, 28–30(–35) × 5–5.5 µm, 4-sterigmatibus. Basidiosporae hyalinae in 3% solutione KOH, globosae vel subglobosae, echinulatae, 4.5–5.5 µm.

HOLOTYPUS: *Italy, Ravenna, Pineta S. Vitale, 15.XI. 2000, A. Bernicchia (7345) in herbarium HUBO concervatus est.*

ETYMOLOGY: the name derives from the shining colour of the fresh material.

ITS rDNA sequence accession numbers—EMBL AJ410780; UNITE UDB000204.

BASIDIOCARP annual, resupinate, separable or rarely adherent to the substratum, pelliculose to arachnoid, continuous. HYMENOPHORE smooth, yellow to orange coloured when fresh, with brick red shades in mature specimens, dull yellow when dry, concolorous with or turning paler than subiculum. STERILE MARGIN smooth to byssoid, yellow, becoming orange coloured in mature specimens. RHIZOMORPHAE not seen, but very thin, slender and yellow mycelial cords sometimes present.

SUBICULAR HYPHAE hyaline or yellowish to very pale brown in 3% KOH, thin-walled to very slightly thick-walled, simple-septate, cross-shaped branching common, 4.2–5.5 µm diam, encrusted with yellow crystals. SUBHYMENIAL HYPHAE hyaline in 3% KOH, simple-septate, thin-walled, smooth, 2.8–4.2 µm diam. CYSTIDIA not seen. CYSTIDIOLES obtuse, flexuose. BASIDIA hyaline, simple-septate at base, 28–30(–35) × 5–5.5 µm, sometimes with numerous

oil drops, 4 sterigmata, slender and 3–4 µm long. Basidiospores hyaline in 3% KOH, yellowish in mass, globose to subglobose, echinulate, 4.5–5.5 µm including spines. Encrustations appear yellow to reddish and better seen in mature specimens, but rapidly dissolve in KOH. Chlamydospores not seen.

Distribution and ecology. So far *T. pulchella* is found only in St. Vitale pine-forest. It lies along the Adriatic coast, about 10–15 km North of Ravenna, and covers at present ca 20 km^2, but coverage has been considerably decreased. This pinewood is a heterogeneous forest, the result of a balance between natural environmental conditions and anthropic influence over many centuries. At the present we can distinguish a xerophilous forest (mainly consisting of *Quercus robur, Q. pubescens, Fraxinus ornus, Pyrus communis, Cornus mas, Acer campestre, Pinus pinea, P. pinaster* and some species of shrubs) on the top of ancient dunes, and the hygrophilous forest established in the depressions between the dunes (with *Populus alba, Fraxinus oxycarpa, Ulmus campestris, Salix alba, S. caprea, Cornus sanguinea* and *Crataegus monogyna*). If *T. pulchella* is an EcM forming fungus then the potential hosts are *Quercus* and / or *Pinus*.

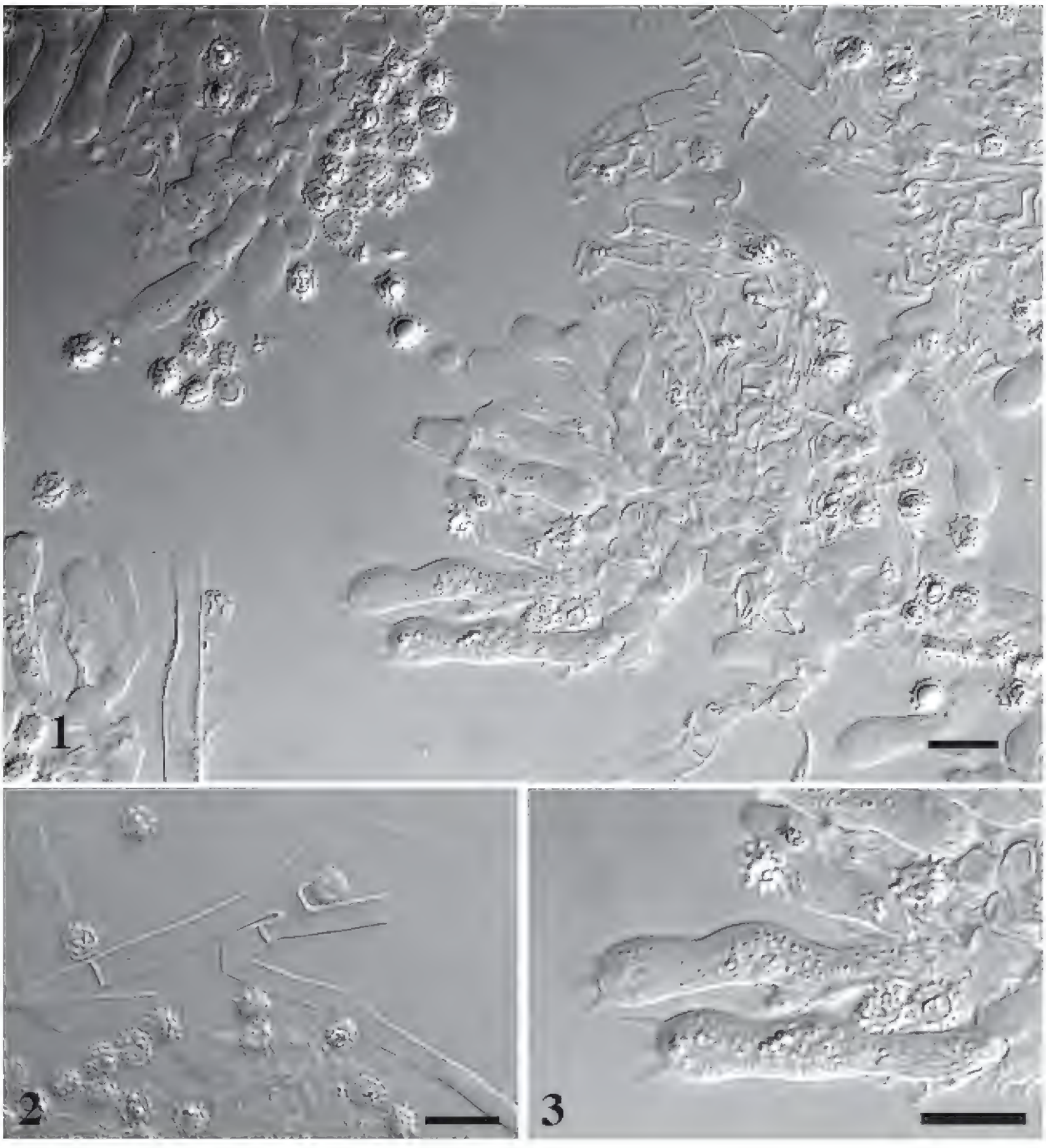
Molecular ecology studies of mycorrhizal fungi have revealed the EcM status of *T. submollis, T. zygodesmoides* and *T. echinospora* (see the placement of EcM ITS sequences on Fig. 4). No EcM ITS sequences have fallen into the cluster with sequences of *T. bresadolana, T. pulchella* and undescribed species. The most likely explanation for this is that these species are comparatively rare and that plant communities where they grow are still not sampled for EcM studies.

Specimens studied – Italy, Ravenna, Pineta St. Vitale, with *Quercus robur, Q. pubescens, Populus alba, Fraxinus ornus, F. oxycarpa, Ulmus campestris, Salix alba, S. caprea, Cornus mas, Pinus pinea, P. pinaster* and *Crataegus monogyna*. Sporocarp collected under dead, fallen branch of *Crataegus monogyna*, 15 November 2000, leg. A. Bernicchia 7345 (HUBO, **holotype**; TU, **isotype**).

Paratypes in HUBO: Pineta St. Vitale (Ravenna), 15.XI.2000, 7344 on *Quercus*, 7855 on litter, 7856 on *Quercus*, 7857 on *Pinus pinea*, 7858 on *Quercus* and *Hedera*, 7859 on *Quercus*, 7860 on *Crataegus* and litter, 5861 on *Quercus*, 7863 on *Crataegus*; 08.X.2001, 7591 on *Crataegus*; 14.X.2001, 7651 on *Crataegus*, 7652 on *Crataegus*.

Remarks. *T. pulchella* is easily distinguished from known species of the same genus by the yellow to orange colour of the hymenium. Two species that in molecular analyses cluster with *T. pulchella* (Fig. 4) have quite different colour. *T. bresadolana* has a lemon-yellow to green hymenium and an undescribed species from Finland has a whitish hymenium. The spores of the new species are also smaller than in *T. bresadolana*. The last taxon has subglobose spores while *T. pulchella* has mostly globose spores.

However, microscopically the species most similar to *T. pulchella* is *T. pusilla* (Hjortstam 1974). The type material of *T. pusilla* is small and collected in 1969. Therefore we did not take a chance to extract DNA from it. There are a few minor differences like colour of basidioma, size of the basidia, etc.



Figs 1–3. ***Tomentellopsis pulchella*** (HUBO 7345, holotype): **1.** Spores, basidia and hyphae; **2.** Spores and anastomosis of hyphae; **3.** Basidia. Bars = 10 μm.

between these two species but above all spores are distinctly dissimilar. The size of the spores is nearly identical, 4.6–5.5 μm in *T. pusilla* and 4.5–5.5 μm in *T. pulchella,* but the shape of the spores is different. *T. pusilla* spores are in lateral view elliptical and in frontal view clearly subglobose while *T. pulchella* spores are in all views globose or slightly subglobose. Also, *T. pusilla* spores have much shorter spines than *T. pulchella.*

The status of *T. pulchella* as a separate species is also supported by the ITS sequence pairwise base differences (Table 1). The closest taxon is *T. bresadolana,* which ITS sequences differ from the *T. pulchella* sequence by

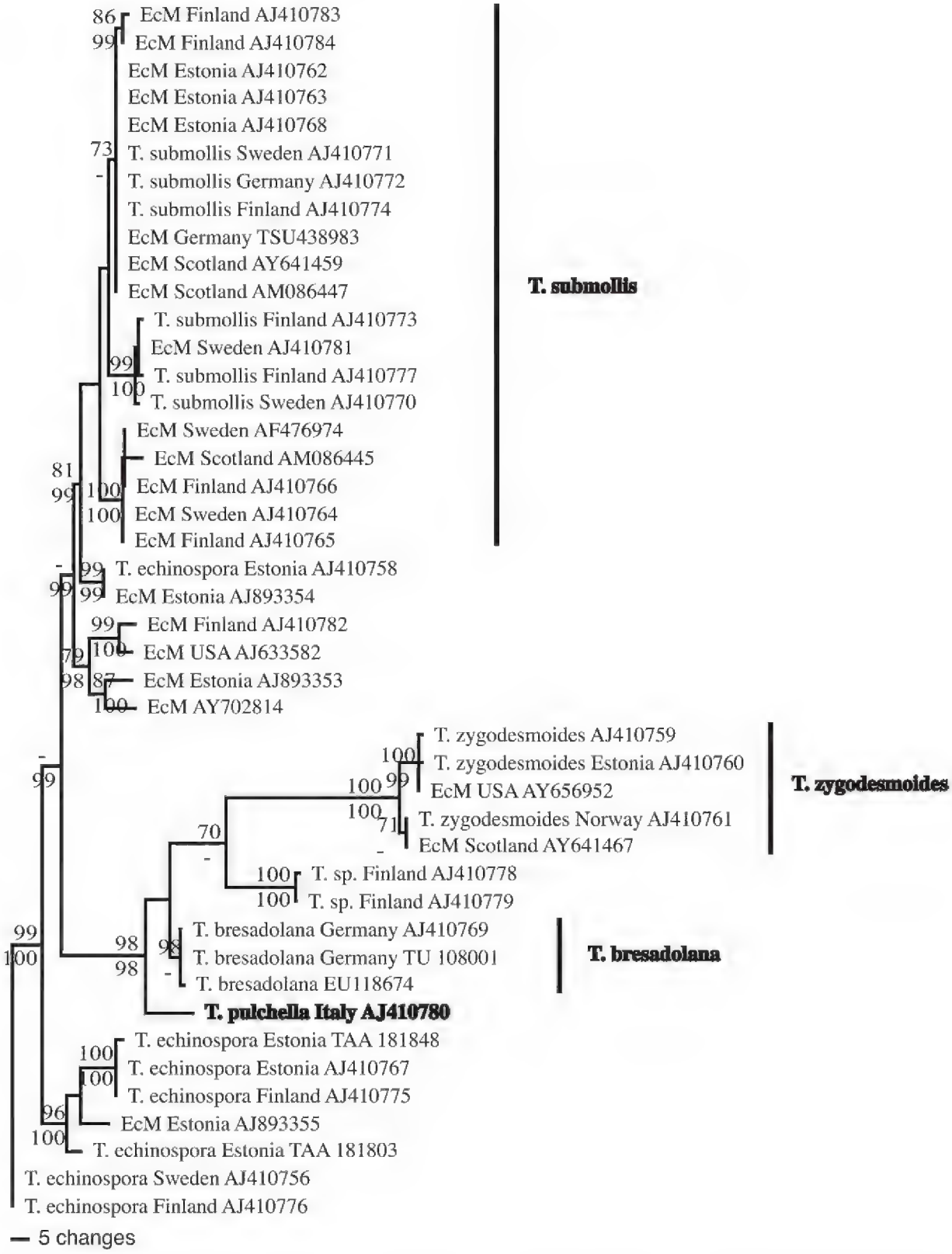


Fig 4. One of the best trees (–ln L = 2392.21980, length = 308, CI = 0.675, RI = 0.890) among 100 equally parsimonious trees depicted as a phylogram. Bootstrap support (≥70%) and posterior probabilities (≥95%) are shown above and below branches.

5.13–5.36%. ITS sequence pairwise base differences are often used in EcM studies for species discrimination. The most commonly used species threshold value is 3% pairwise base difference (e.g. Tedersoo et al. 2003).

Table 1. rDNA ITS sequence pairwise base differences of three *Tomentellopsis* species.

Species pair	Proportion of site differences	Pairwise base difference
T. pulchella versus *T. bresadolana* AJ410796	26/485	5.36%
T. pulchella versus *T. bresadolana* EU118674	27/507	5.33%
T. pulchella versus *T. bresadolana* TU 108001	26/507	5.13%
T. pulchella versus *T. sp.* AJ410778	43/486	8.85%
T. pulchella versus *T. sp.* AJ410779	44/488	9.02%

Kõljalg et al. (2002) did not accept *T. pulchella* as different from *T. bresadolana*. However, they stressed that ITS sequences of this group deviate from each other by 8–9% and therefore probably included two or more species. At that time only one sequence from a well-developed basidioma of *T. bresadolana* was available. In the present study we added two new ITS sequences of *T. bresadolana* from Sweden and Germany, which made the division of the group into three separate species clear. The undescribed *Tomentellopsis* species collected in Finland will be validly published in a forthcoming treatment of the genus.

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References

Bernicchia A. 2005. *Polyporaceae* s.l. Fungi Europaei 10. Edizioni Candusso, Italia.

Burke DJ, Martin KJ, Rygiewicz PT, Topa MA. 2005. Ectomycorrhizal fungi identification in single and pooled root samples: terminal restriction fragment length polymorphism (TRFLP) and morphotyping compared. Soil Biology and Biochemistry 37: 1683–1694.

Gardes M, Bruns TD. 1993. ITS primers with enhanced specificity for basidiomycetes – application to the identification of mycorrhizae and rusts. Molecular Ecology 2: 113–118.

Hausknecht A, Zuccherelli A. 1999. *Porpoloma juncicola*, eine neue Art aus Ravenna (Italien). Österreichische Zeitschrift für Pilzkunde 8: 153–155.

Hjortstam K. 1974. Studies in the Swedish Species of the Genus *Tomentella* (*Thelephoraceae*) III. The genus *Tomentellopsis*. Svensk Botanisk Tidskrift 68: 51–56.

Huelsenbeck JP, Ronquist F. 2001. MRBAYES: Bayesian inference of phylogenetic trees. Bioinformatics 17: 754–755.

Kõljalg U. 1996. *Tomentella* and related genera in temperate Eurasia. Synopsis Fungorum 9. Fungiflora, Oslo.

Kõljalg U, Tammi H, Timonen S, Agerer R, Sen R. 2002. ITS rDNA nucleotide sequence-based phylogenetic analysis of *Tomentellopsis* species from boreal and temperate forests, and the identification of pink-type ectomycorrhizas. Mycological Progress 1: 81–92.

Kõljalg U, Larsson K-H, Abarenkov K, Nilsson RH, Alexander IJ, Eberhardt U, Erland S, Hoiland K, Kjoller R, Larsson E, Pennanen T, Sen R, Taylor AFS, Tedersoo L, Vralstad T, Ursing BM. 2005. UNITE: a database providing web-based methods for the molecular identification of ectomycorrhizal fungi. New Phytologist 166: 1063–1068.

Noordeloos ME, Hausknecht A. 2002. Weitere neue *Entoloma*-Arten aus Südeuropa. Österreichische Zeitschrift für Pilzkunde 11: 117–131.

Nylander JAA. 2004. MrModeltest v2.2. Available at http://www.abc.se/~nylander/.

Posada D, Crandall KA. 1998. Modeltest: testing the model of DNA substitution. Bioinformatics 14: 817–818.

Rosling A, Landeweert R, Lindahl BD, Larsson K-H, Kuyper TW, Taylor AFS, Finlay RD. 2003. Vertical distribution of ectomycorrhizal fungal taxa in a podzol soil profile. New Phytologist 159: 775–783.

Saari SK, Campbell CD, Russell J, Alexander IJ, Anderson IC. 2005. Pine microsatellite markers allow roots and ectomycorrhizas to be linked to individual trees. New Phytologist 165: 295–304.

Swofford DL. 2003. PAUP*: Phylogenetic Analysis Using Parsimony (*and Other Methods). Version 4. Sinauer Associates, Sunderland, Massachusetts.

Tedersoo L, Kõljalg U, Hallenberg N, Larsson K-H. 2003. Fine scale distribution of ectomycorrhizal fungi and roots across substrate layers including coarse woody debris in a mixed forest. New Phytologist 159: 153–165.

Tedersoo L, Suvi T, Larsson E, Kõljalg U. 2006. Diversity and community structure of ectomycorrhizal fungi in a wooded meadow. Mycological Research 110: 734–748.

Walker JF, Miller OK, Horton JL. 2005. Hyperdiversity of ectomycorrhizal fungus assemblages on oak seedlings in mixed forests in the southern Appalachian Mountains. Molecular Ecology 14: 829–838.

White TJ, Bruns T, Lee S, Taylor J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics: 315–322. In: MA Innis et al. (eds.), PCR protocols: A guide to methods and applications. San Diego, Academic Press.